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CITATION:

Hou, Zengye ...[et al]. Diversity-oriented synthesis of pyrazolo[4,3-b]indoles by gold-catalysed three-component annulation: application to the development of a new class of CK2 inhibitors.. Organic & biomolecular chemistry 2013, 11(20): 3288-3296

ISSUE DATE:

2013-05-28

URL:

<http://hdl.handle.net/2433/189820>

RIGHT:

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Diversity-oriented synthesis of pyrazolo[4,3-*b*]indoles by gold-catalysed three-component annulation: application to the development of a new class of CK2 inhibitors

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Abstract: Pyrazolo[4,3-*b*]indole derivatives have been designed as novel CK2 inhibitor compounds based on the binding mode analysis of a previously reported phenylpyrazole-type CK2 inhibitor. A series of pyrazolo[4,3-*b*]indoles and related dihydropyrazolo[4,3-*b*]indoles were efficiently prepared from simple starting materials using a gold-catalysed three-component annulation reaction as a key step. Several of the newly synthesized compounds displayed high levels of inhibitory activity, indicating that the pyrazolo[4,3-*b*]indole core represents a promising scaffold for the development of potent CK2 inhibitors.

Introduction

The protein kinase CK2 (previously known as casein kinase II) is a ubiquitous, essential and highly pleiotropic serine/threonine specific kinase with hundreds of endogenous substrates that are implicated in a wide variety of different cellular functions.¹ CK2 typically forms tetrameric complexes consisting of two catalytic α subunits (i.e. α or α') and two regulatory β subunits in a variety of different combinations.² The CK2 α' subtype is exclusively expressed in the brain and testis,

whereas the CK2 α subtype has been reported to be ubiquitously expressed.³ A significant body of evidence is available within the literature to support the idea that CK2 behaves in multifunctional capacity in a number of cellular events contributing to the progression of cancer, making it a potential target for cancer treatment.⁴ Although many ATP-competitive and ATP non-competitive CK2 inhibitors have already been reported in the literature,⁵ the benzonaphthyridine derivative CX-4945 (**1**) is the only one of these inhibitors to have reached clinical trials for the treatment of cancer (Figure 1).^{5g,5h}

In our initial efforts to develop novel CK2 inhibitors, we performed a series of structure–activity relationship studies^{6a} and crystallographic analyses⁷ of pyrazine-based CK2 inhibitors **2**^{6b,c} (Figure 1). These studies revealed that these inhibitors **2** bind to the ATP-binding site with a planar horseshoe-shaped conformation and that the nitrogen atom at the 4-position of the pyrazine ring and the (pyrrol-3-yl)acetic acid moiety were necessary for the binding affinities. Furthermore, the virtual screening of a compound library using the structure of the CK2 α –**2b** complex, led to the identification of a novel CK2 inhibitor lead compound **3a**, which consisted of a phenylpyrazole scaffold (Figure 1).⁸ Binding mode analysis suggested that inhibitor **3** could tightly bind to the ATP-binding region through a series of favourable interactions, whereas the other tautomer **3'**, in which the NH and N positions on the pyrazole ring had been swapped around, could give rise to unfavourable electrostatic repulsions with the CK2 α (Figure 2). Based on this structural information, it was envisaged that the inhibitory activity of the lead compound **3a** could be improved by controlling the tautomerization process and pushing it towards the favoured structure. With this in mind, we designed and synthesized a series of benzo[g]indazole derivatives **4** (Figure 1)⁹ as novel CK2 inhibitor compounds. The compounds themselves could be regarded as a conformationally restricted analogue of the phenylpyrazole **3**. It was anticipated that restricting the rotatable bond in this way would provide the preferred planar conformation and lead to a reduction in the entropic loss encountered during the binding to CK2 α . Furthermore, it was envisaged that the desired tautomer of

the pyrazole moiety would be formed preferentially because of the presence of the fused benzene ring (Figure 3).¹⁰ Indeed, the synthesized benzo[*g*]indazole derivatives **4a** [$IC_{50} = 0.040 \mu M$ (CK2 α) and $0.042 \mu M$ (CK2 α')] and **4b** [$IC_{50} = 0.089 \mu M$ (CK2 α) and $0.067 \mu M$ (CK2 α')] exhibited higher levels of inhibitory activity towards CK2 than the phenylpyrazole **3a** [$IC_{50} = 0.14 \mu M$ (CK2 α) and $0.063 \mu M$ (CK2 α')] (Figure 1). In the present work, we have designed and synthesized a series of pyrazolo[4,3-*b*]indole derivatives **5** (Figure 3) as a new class of CK2 inhibitor compounds in which the phenylpyrazole moiety was bridged with a nitrogen atom. Based on the same discussion provided for the development of the benzo[*g*]indazole-type inhibitors **4**, enhanced levels of inhibitory activity were also expected for the pyrazolo[4,3-*b*]indoles **5**.

Several methods have been described in the literature for the synthesis of pyrazoloindoles according to linear synthetic strategies.¹¹ To the best of our knowledge, however, there have been no reports describing the synthesis of pyrazoloindoles based on multiple component reactions (MCRs).¹² MCRs provide a divergent approach to functionalised pyrazoloindoles from simple starting materials. We have recently developed a novel gold-catalysed three-component annulation reaction of alkynes, hydrazines and aldehydes/ketones for the direct synthesis of polysubstituted dihydropyrazoles (Scheme 1).¹³ The mechanism of this reaction involves the formation of the propargyl hydrazine intermediate via the Mannich-type coupling of alkynes with *N,N'*-disubstituted hydrazines and aldehydes/ketones, followed by intramolecular hydroamination. It was envisaged that the pyrazolo[4,3-*b*]indole-type inhibitor compounds **5** could be efficiently synthesized using this gold-catalysed three-component annulation reaction. Herein, we report our synthetic studies towards the pyrazolo[4,3-*b*]indole derivatives **5** and their subsequent evaluation as CK2 inhibitors.

Results and discussion

Our initial efforts were focused on designing a strategy for the construction of pyrazolo[4,3-*b*]indole scaffold via a diversity-oriented synthetic route (Scheme 2). The multi-substituted

pyrazolo[4,3-*b*]indoles **11** could be directed from their dihydropyrazolo[4,3-*b*]indole congeners **10** via an oxidative aromatization reaction. The dihydropyrazolo[4,3-*b*]indoles **10** themselves could in principle be accessed by the intramolecular C-H amination of the corresponding dihydropyrazoles **9** substituted with a phenylazide moiety. It is important to point out at this stage, however, that this transformation represented an unprecedented process¹⁴ during the design of our synthetic strategy. It was anticipated that the dihydropyrazoles **9** could be synthesized by the gold-catalysed three-component annulation of the ethynylbenzene derivatives **6** bearing an azide group at their *ortho*-positions with a variety of different aldehydes and disubstituted hydrazines.

The gold-catalysed three-component annulation was initially investigated using 1-azido-2-ethynylbenzene (**6a**), isobutyraldehyde (**7a**) and hydrazine derivatives **8a** bearing a 4-methoxybenzyl (PMB) group as model substrates (Scheme 3). Under the standard reaction conditions [i.e. 3 mol % of IPrAuCl/AgOTf in 1,2-dichloroethane (1,2-DCE)] developed during a previously reported optimization process,¹³ the Mannich-type coupling and cyclization reactions proceeded smoothly to afford the dihydropyrazole **9a** in 73% yield.¹⁵ A number of transition metal complexes and Lewis acids have been reported to promote C-N bond formation reactions from aryl azides.¹⁴ Of the various catalysts reported, ruthenium(III) chloride hydrate (RuCl₃·nH₂O)^{14e,14f} was found to facilitate the intramolecular C-H amination reaction to provide the desired dihydropyrazolo[4,3-*b*]indole **10a** in moderate yield (62%). Subsequent treatment of **10a** with ceric ammonium nitrate (CAN) facilitated the cleavage of PMB protecting group and the aromatization of the dihydropyrazole ring to give the pyrazolo[4,3-*b*]indole **11a** in high yield (87%).

Having established the standard protocol to access the desired scaffold, we intended to determine its scope and limitations using other ethynylbenzenes, hydrazines, and aldehydes (or ketones). Thus, we proceeded to examine the synthesis of multi-substituted dihydropyrazolo[4,3-*b*]indoles **10** (and/or pyrazolo[4,3-*b*]indoles **11**) via the three-component annulation reaction followed by an intramolecular C-H amination (Table 1). The introduction of electron-withdrawing

and electron-donating groups to the *para*-position of the phenyl ring of the ethynylbenzene unit was well tolerated (Table 1, entries 2 and 3). Considering the further application of this synthetic strategy to the synthesis of the CK2 inhibitor compounds **5**, which contain a carboxy group, ethyl 3-azido-4-ethynylbenzoate **6b** was used as the ethynylbenzene component throughout the remainder of the investigation. The use of hydrazines bearing *tert*-butoxycarbonyl (Boc) and benzyl (Bn) groups was also successful (Table 1, entry 4). When other aliphatic and aromatic aldehydes were tested, modifications to the reaction conditions were required, with silver bis(trifluoromethanesulphonyl)amide (AgNTf₂) being used as the silver salt instead of AgOTf to effectively promote the three-component annulation reactions (Table 1, entries 5–10). When *p*-anisaldehyde was used as the aldehyde component, the efficiency of the three-component annulation was significantly diminished, with the desired product **9i** being isolated in low yield (31%, Table 1, entry 9). It is noteworthy that the intramolecular C-H amination of the dihydropyrazole **9g** prepared from benzaldehyde gave a mixture of dihydropyrazolo[4,3-*b*]indole **10g** and its aromatized product **11b** (Table 1, entry 7). Particularly for the case of dihydropyrazole **9i** prepared from *p*-anisaldehyde, pyrazolo[4,3-*b*]indole **11c** was isolated as the single product (Table 1, entry 9). As the use of the ketone, cyclohexanone instead of an aldehyde was well tolerated under the reaction conditions of the three-component annulation, and afforded the dihydropyrazole **9j** in good yield (Table 1, entry 10). The intramolecular C-H amination of **9j** in the presence of the RuCl₃·*n*H₂O catalyst failed, whereas the application of traditional thermal decomposition conditions^{14j} to **9j** gave the desired dihydropyrazolo[4,3-*b*]indole **10i** in moderate yield (Table 1, entry 10). Disappointingly, the three-component annulation using paraformaldehyde provided none of the desired product **9k**, and resulted instead in the formation of a complex mixture (Table 1, entry 11).

For the synthesis of dihydropyrazole **9k**, the decision was taken to adopt a step-wise synthetic procedure (Scheme 4) because of difficulties encountered during the attempted synthesis of this compound under the three-component annulation conditions. The Sonogashira coupling reaction of

the aryl iodide derivative **12** with the propargyl hydrazine derivative **13** afforded the cyclization precursor **14**. Subsequent heating of **14** in the presence of a catalytic amount of IPrAuCl/AgOTf in 1,2-DCE facilitated the necessary 5-*endo-dig* cyclization process to furnish the desired dihydropyrazole **9k** in good yield (74%). Under the thermal decomposition conditions, **9k** was successfully converted to the dihydropyrazolo[4,3-*b*]indole **10j** in modest yield (43%).

With a verity of different dihydropyrazolo[4,3-*b*]indole derivatives in hand, we proceeded with the preparation of the pyrazolo[4,3-*b*]indole-type CK2 inhibitor compounds **5a–g** using further functional group transformations (Scheme 5). The *N*-substituted dihydropyrazolo[4,3-*b*]indole derivatives **15a–d** were readily obtained via the *N*-alkylation or *N*-arylation reactions of the dihydropyrazolo[4,3-*b*]indole derivatives **10b**, **10g** and **10j**. Subsequent treatment of **10b**, **10g**, **10j** and **15a–d** with CAN affected the required oxidative aromatization reactions to give the pyrazolo[4,3-*b*]indoles **11b** and **11d–i**. For the final step in the synthesis, the methyl carbamate and ester protecting groups were removed to yield the desired compounds **5a–g**.

We then investigated the *in vitro* inhibitory activities of the synthesized derivatives toward CK2 α and CK2 α' (Table 2). The analogues **5b** and **5f** bearing an isopropyl group on their pyrazole ring ($R^1 = i\text{-Pr}$) showed no inhibition against CK2 at 1 μM . In contrast, the benzene-substituted analogues **5c** and **5g** ($R^1 = \text{Ph}$) exhibited relatively high levels of inhibitory activity. For the 3-unsubstituted derivatives **5a**, **5d** and **5e** ($R^1 = \text{H}$), the presence of some degree of substitution on the nitrogen atom of the indole moiety (R^2) was found to be crucial to the high binding affinities. The introduction of a Bn group on the nitrogen atom ($R^2 = \text{Bn}$) led to a reduction in the inhibitory activity, whereas the introduction of a 4-nitrophenyl group at the same position resulted in a 10-fold increase in the potency. Of all of the compounds synthesised, compounds **5c** and **5e** displayed almost equipotent inhibitory activities to the lead compound **3a** and benzo[*g*]indazole **4b**. The introduction of an aromatic ring at the R^1 or R^2 position led to enhanced binding affinities, perhaps because it could form hydrophobic interactions with the surrounding amino acid residues of CK2 α .¹⁶

Considering the loss of the amino group from the pyrazole ring of these compounds, which would otherwise have formed a favourable hydrogen bonding interaction with the carbonyl oxygen of the Val116 residue (Figure 2), the pyrazolo[4,3-*b*]indole core represents a promising scaffold for the development of potent CK2 inhibitors.

Conclusion

In conclusion, we have designed a series of pyrazolo[4,3-*b*]indole derivatives as novel CK2 inhibitor compounds on the basis of our previous studies on a phenylpyrazole-based CK2 inhibitor. Diversity-oriented synthesis of the desired pyrazolo[4,3-*b*]indoles and related dihydropyrazolo[4,3-*b*]indoles was achieved by the application of our gold-catalysed three-component annulation reaction. Evaluation of the CK2 inhibitory activities of the resulting compounds led to the identification of several potent inhibitors.

Experimental section

General

Melting points were measured by a hot stage melting point apparatus (uncorrected). ¹H NMR spectra were recorded using a JEOL ECA-500 spectrometer and chemical shifts are reported in δ (ppm) relative to TMS as internal standard. ¹³C NMR spectra were recorded using a JEOL ECA-500 spectrometer and referenced to the residual solvent signal. ¹H NMR spectra are tabulated as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br s = broad singlet), coupling constant(s) and number of protons. Exact mass (HRMS) spectra were recorded on a JMS-HX/HX 110A mass spectrometer. Infrared (IR) spectra were obtained on a JASCO FT/IR-4100 FT-IR spectrometer with JASCO ATR PRO410-S. The purity of the compounds **5a–g** was determined as $\geq 95\%$ by HPLC (method A: Cosmosil 5C18-ARII column, 250 mm \times 4.6 mm; water/acetonitrile containing 0.1% TFA; linear gradient from 90:10 to 70:30 over 30 min; flow

rate of 1 cm³/min; UV detector at 220 nm or method B: Cosmosil 5C18-ARII column, 250 mm × 4.6 mm; water/acetonitrile containing 0.1% TFA; linear gradient from 70:30 to 50:50 over 30 min; flow rate of 1 cm³/min; UV detector at 220 nm). Compounds **8a**⁹ and **8b**¹³ were prepared according to the literature.

Representative procedure for gold-catalysed three-component annulation of 6a–c with 7 and 8a,b: synthesis of methyl 5-(2-azidophenyl)-3-isopropyl-2-(4-methoxybenzyl)-2,3-dihydro-1H-pyrazole-1-carboxylate (9a). Under argon atmosphere, the mixture of **6a** (72 mg, 0.5 mmol), hydrazine **8a** (158 mg, 0.75 mmol), isobutyraldehyde **7a** (0.068 cm³, 0.75 mmol), IPrAuCl (9.3 mg, 0.015 mmol) and AgOTf (3.9 mg, 0.015 mmol) in 1,2-dichloroethane (2.5 cm³) was stirred at 50 °C for 4 h. After being cooled to room temperature, the mixture was filtered through Celite and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc = 5/1) to afford the title compound **9a** (149 mg, 73%) as a colourless oil: IR (neat): $\nu_{\text{max}}/\text{cm}^{-1}$ 2125 (N₃), 1708 (C=O); δ_{H} (500 MHz, CDCl₃) 0.75–0.77 (6H, m), 1.51–1.58 (1H, m), 3.35 (1H, dd, *J* 5.7, 2.9 Hz), 3.59 (3H, s), 3.77 (1H, d, *J* 12.0 Hz), 3.80 (3H, s), 4.09 (1H, d, *J* 12.0 Hz), 5.42 (1H, d, *J* 2.9 Hz), 6.86 (2H, d, *J* 8.6 Hz), 7.10–7.13 (1H, m), 7.17–7.18 (1H, m), 7.32–7.36 (2H, m), 7.38 (2H, d, *J* 8.6 Hz); δ_{C} (125 MHz, CDCl₃) 18.0, 18.2, 32.8, 52.6, 55.2, 61.6, 73.6, 112.8, 113.3 (2C), 118.3, 124.3, 124.7, 129.0, 129.3, 130.2, 131.5 (2C), 137.2, 137.7, 155.0, 159.0; HRMS (FAB⁺) Calcd for C₂₂H₂₆N₅O₃ [*M*+H]⁺: 408.2036, found: 408.2030.

Representative procedure for ruthenium-catalysed intramolecular C-H amination of 9a–i: synthesis of methyl 3-isopropyl-2-(4-methoxybenzyl)-1,2,3,4-tetrahydropyrazolo[4,3-*b*]indole-1-carboxylate (10a). Under argon atmosphere, the mixture of **9a** (122 mg, 0.3 mmol) and RuCl₃·nH₂O (3.9 mg, 0.015 mmol) in 1,2-dimethoxyethane (3 cm³) was stirred at 80 °C for 4 h. After being cooled to room temperature, the mixture was filtered through Celite and the filtrate was concentrated

in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc = 3/1) to afford the title compound **10a** (71 mg, 62%) as a colourless oil: IR (neat): $\nu_{\max}/\text{cm}^{-1}$ 1693 (C=O); δ_{H} (500 MHz, CDCl_3) 0.70–0.75 (6H, m), 1.68–1.74 (1H, m), 3.79–3.83 (5H, m), 3.87 (3H, s), 4.22 (1H, d, J 12.0 Hz), 6.84 (2H, d, J 8.6 Hz), 7.12–7.17 (2H, m), 7.29–7.31 (1H, m), 7.34 (2H, d, J 8.6 Hz), 7.83–7.86 (2H, m); δ_{C} (125 MHz, CDCl_3) 17.7, 18.4, 33.2, 52.9, 55.2, 62.4, 69.9, 111.9, 113.4 (2C), 117.2, 119.6, 120.3, 121.8, 123.8, 128.7, 129.7, 131.4 (2C), 140.2, 154.4, 159.1; HRMS (FAB⁺) Calcd for $\text{C}_{22}\text{H}_{26}\text{N}_3\text{O}_3$ [$M+\text{H}$]⁺: 380.1974, found: 380.1978.

Representative procedure for removal of PMB group and simultaneous aromatization of fused dihydropyrazoles 10a, 10b, 10j and 15a–d: synthesis of methyl 3-isopropyl-1,4-dihydropyrazolo[4,3-*b*]indole-1-carboxylate (11a). The mixture of **10a** (60 mg, 0.16 mmol) and CAN (175 mg, 0.32 mmol) in MeCN (1.8 cm³) and H₂O (0.2 cm³) was stirred at room temperature for 12 h. The resulting mixture was diluted with H₂O and extracted with EtOAc twice. The combined extracts were washed with brine, dried over Na₂SO₄ and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc = 3/1) to afford the title compound **11a** (36 mg, 87%) as a white solid: mp 183 °C; IR (neat): $\nu_{\max}/\text{cm}^{-1}$ 1742 (C=O); δ_{H} (500 MHz, CDCl_3) 1.46 (6H, d, J 6.9 Hz), 3.28–3.35 (1H, m), 4.16 (3H, s), 7.21–7.25 (1H, m), 7.34–7.44 (2H, m), 7.95–8.00 (1H, m), 8.28 (1H, br s); δ_{C} (125 MHz, CDCl_3) 21.7 (2C), 27.9, 54.6, 112.3, 114.5, 120.2, 121.6, 125.4, 129.5, 133.1, 143.5, 147.5, 150.7; HRMS (FAB⁺) Calcd for $\text{C}_{14}\text{H}_{16}\text{N}_3\text{O}_2$ [$M+\text{H}$]⁺: 258.1243, found: 258.1238.

Methyl 2-(4-methoxybenzyl)-2-(prop-2-ynyl)hydrazinecarboxylate (13). To a stirred mixture of hydrazine **8a** (105 mg, 0.5 mmol) and K₂CO₃ (138 mg, 1 mmol) in DMF (1 cm³) was added propargyl bromide (0.045 cm³, 0.6 mmol) and the resulting mixture was stirred at 40 °C for 12 h. After being cooled to room temperature, the mixture was diluted with H₂O and extracted with

EtOAc twice. The combined extracts were washed with brine, dried over Na₂SO₄ and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc = 4/1) followed by recrystallization from EtOAc–hexane to afford the title compound **13** (105 mg, 85%) as colourless crystals: mp 71 °C; IR (neat): $\nu_{\max}/\text{cm}^{-1}$ 3227 (C≡CH), 1714 (C=O); δ_{H} (500 MHz, CDCl₃) 2.37 (1H, t, *J* 2.3 Hz), 3.59 (2H, br s), 3.66 (3H, s), 3.79 (3H, s), 3.86 (2H, br s), 5.99 (1H, br s), 6.85 (2H, d, *J* 8.6 Hz), 7.30 (2H, d, *J* 8.6 Hz); δ_{C} (125 MHz, CDCl₃) 45.3, 52.3, 55.1, 59.6, 61.4, 74.9, 113.7 (2C), 127.7, 130.6 (2C), 155.7, 159.2; HRMS (FAB⁺) Calcd for C₁₃H₁₇N₂O₃ [*M*+H]⁺: 249.1239, found: 249.1235.

Methyl 2-{3-[2-azido-4-(ethoxycarbonyl)phenyl]prop-2-ynyl}-2-(4-methoxybenzyl)hydrazinecarboxylate (14). Under argon atmosphere, the mixture of iodobenzene **12** (103 mg, 0.33 mmol), propargyl hydrazine **13** (97 mg, 0.39 mmol), Pd(PPh₃)₂Cl₂ (11 mg, 0.016 mmol), CuI (3.1 mg, 0.016 mmol) and Et₃N (0.23 cm³, 1.63 mmol) in THF (2 cm³) was stirred at 40 °C for 5 h. After being cooled to room temperature, the mixture was filtered through Celite and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc = 3/1) to afford the title compound **14** (100 mg, 70%) as a brown oil: IR (neat): $\nu_{\max}/\text{cm}^{-1}$ 2251 (C≡C), 2115 (N₃), 1718 (C=O); δ_{H} (500 MHz, CDCl₃) 1.42 (3H, t, *J* 7.2 Hz), 3.68 (3H, s), 3.79 (3H, s), 3.86 (2H, s), 3.97 (2H, br s), 4.41 (2H, q, *J* 7.2 Hz), 6.40 (1H, br s), 6.87 (2H, d, *J* 8.6 Hz), 7.35 (2H, d, *J* 8.6 Hz), 7.48 (1H, d, *J* 8.0 Hz), 7.76–7.78 (1H, m), 7.83 (1H, s); δ_{C} (125 MHz, CDCl₃) 14.2, 46.3, 52.3, 55.2, 60.1, 61.6, 82.3, 91.4, 113.8 (2C), 118.7, 119.2, 125.5, 127.8, 130.7 (2C), 131.5, 133.1, 141.9, 155.8, 159.3, 165.1; HRMS (FAB⁺) Calcd for C₂₂H₂₄N₅O₅ [*M*+H]⁺: 438.1777, found: 438.1781.

Methyl 5-[2-azido-4-(ethoxycarbonyl)phenyl]-2-(4-methoxybenzyl)-2,3-dihydro-1H-pyrazole-1-carboxylate (9k). Under argon atmosphere, the mixture of **14** (95 mg, 0.22 mmol),

IPrAuCl (4.3 mg, 0.007 mmol) and AgOTf (1.8 mg, 0.007 mmol) in 1,2-dichloroethane (1 cm³) was stirred at 50 °C for 4 h. After being cooled to room temperature, the mixture was filtered through Celite and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc = 3/1) to afford the title compound **9k** (70 mg, 74%) as a yellow oil: IR (neat): $\nu_{\max}/\text{cm}^{-1}$ 2114 (N₃), 1714 (C=O); δ_{H} (500 MHz, CDCl₃) 1.40 (3H, t, *J* 7.2 Hz), 3.59 (3H, s), 3.80–3.82 (5H, m), 3.95 (2H, br s), 4.40 (2H, q, *J* 7.2 Hz), 5.67 (1H, t, *J* 2.3 Hz), 6.89 (2H, d, *J* 9.2 Hz), 7.37–7.39 (3H, m), 7.78–7.80 (1H, m), 7.86 (1H, s); δ_{C} (125 MHz, CDCl₃) 14.3, 52.9, 55.2, 57.3, 61.3, 61.5, 112.6, 113.6 (2C), 119.5, 125.4, 128.3, 128.5, 130.0, 131.2 (2C), 131.4, 136.7, 137.9, 155.0, 159.2, 165.4; HRMS (FAB⁺) Calcd for C₂₂H₂₄N₅O₅ [*M*+H]⁺: 438.1778, found: 438.1776.

6-Ethyl 1-methyl 2-(4-methoxybenzyl)-1,2,3,4-tetrahydropyrazolo[4,3-*b*]indole-1,6-dicarboxylate (10j). Under argon atmosphere, a solution of **9k** (350 mg, 0.8 mmol) in *o*-dichlorobenzene (8 cm³) was stirred at 150 °C for 2 h. After being cooled to room temperature, the resulting mixture was chromatographed on silica gel (hexane/EtOAc = 2/1) to afford the title compound **10j** (141 mg, 43%) as a brown solid: mp 152 °C; IR (neat): $\nu_{\max}/\text{cm}^{-1}$ 1708 (C=O); δ_{H} (500 MHz, CDCl₃) 1.41 (3H, t, *J* 7.2 Hz), 3.78 (3H, s), 3.87 (3H, s), 4.07 (2H, s), 4.23 (2H, br s), 4.39 (2H, q, *J* 7.2 Hz), 6.84 (2H, d, *J* 8.6 Hz), 7.32 (2H, d, *J* 8.6 Hz), 7.82–7.85 (2H, m), 8.06 (1H, s), 8.52 (1H, s); δ_{C} (125 MHz, CDCl₃) 14.4, 52.5, 53.2, 55.2, 60.8, 62.7, 113.8 (2C), 114.3, 118.9, 120.0, 121.1, 121.4, 123.6, 128.1, 131.0 (2C), 131.6, 139.5, 154.6, 159.3, 167.6; HRMS (FAB⁺) Calcd for C₂₂H₂₄N₃O₅ [*M*+H]⁺: 410.1716, found: 410.1711.

Representative procedure for *N*-alkylation or *N*-arylation of 10b, 10j and 10g: synthesis of 6-ethyl 1-methyl 4-benzyl-3-isopropyl-2-(4-methoxybenzyl)-1,2,3,4-tetrahydropyrazolo[4,3-*b*]indole-1,6-dicarboxylate (15c). To a solution of **10b** (39 mg, 0.09 mmol) in DMF (1 cm³) was added NaH (2.6 mg, 0.11 mmol) at room temperature. After being stirred for 30 min, BnBr (0.012

cm³, 0.11 mmol) was added dropwise and then the mixture was stirred for further 30 min. The resulting mixture was diluted with H₂O and extracted with EtOAc twice. The combined extracts were washed with brine, dried over Na₂SO₄ and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc = 5/1) to afford the title compound **15c** (44 mg, 90%) as a yellow oil: IR (neat): $\nu_{\max}/\text{cm}^{-1}$ 1701 (C=O); δ_{H} (500 MHz, CDCl₃) 0.60–0.64 (6H, m), 1.40 (3H, t, *J* 7.1 Hz), 1.57–1.63 (1H, m), 3.58 (1H, d, *J* 3.4 Hz), 3.63 (1H, d, *J* 12.0 Hz), 3.78 (3H, s), 3.89 (3H, s), 4.08 (1H, d, *J* 12.0 Hz), 4.38 (2H, q, *J* 7.1 Hz), 5.11 (1H, d, *J* 16.0 Hz), 5.45 (1H, d, *J* 16.0 Hz), 6.77 (2H, d, *J* 8.6 Hz), 6.96–6.98 (2H, m), 7.10 (2H, d, *J* 8.6 Hz), 7.31–7.32 (3H, m), 7.83–7.85 (2H, m), 8.04 (1H, s); δ_{C} (125 MHz, CDCl₃) 14.4, 16.6, 19.1, 32.8, 48.4, 53.0, 55.2, 60.7, 62.2, 68.2, 112.5, 113.3 (2C), 119.2, 119.8, 121.0, 123.3, 126.6 (2C), 128.0, 128.1, 128.9 (2C), 131.7 (2C), 136.6, 136.7, 139.4, 140.1, 159.1, 167.5, 169.9; HRMS (FAB⁺) Calcd for C₃₂H₃₆N₃O₅ [*M*+H]⁺: 542.2655, found: 542.2661.

Representative procedure for hydrolysis of the methyl carbamate and ester of 11b and 11d–i: synthesis of 1,4-dihydropyrazolo[4,3-*b*]indole-6-carboxylic acid (5a). To a mixture of **11d** (40 mg, 0.14 mmol) in THF (1 cm³), H₂O (0.5 cm³) and MeOH (0.5 cm³) was added 3N NaOH (0.2 cm³) and the mixture was stirred at 60 °C for 5 h. After being cooled to room temperature, the mixture was neutralized with 3N HCl (0.2 cm³). The resulting mixture was chromatographed on silica gel (CHCl₃/MeOH = 9/1) to afford the title compound **5a** (22 mg, 78%) as a white solid: mp >300 °C; IR (neat): $\nu_{\max}/\text{cm}^{-1}$ 1726 (C=O); δ_{H} (500 MHz, DMSO-*d*₆) 7.64 (1H, s), 7.68 (1H, d, *J* 8.4 Hz), 7.83 (1H, d, *J* 8.4 Hz), 8.03 (1H, s), 10.65 (1H, s), 12.30–13.32 (2H, m); δ_{C} (125 MHz, DMSO-*d*₆) 113.7, 114.7, 116.9, 118.5, 119.0, 126.0, 132.3, 136.5, 144.1, 168.0; HRMS (FAB⁺) Calcd for C₁₀H₈N₃O₂ [*M*+H]⁺: 202.0617, found: 202.0612; *t*_R (method A): 14.31 min.

CK2 kinase assay

CK2 inhibitory activities were evaluated by the off-chip mobility shift assay by the QuickScout® service from Carna Bioscience (Kobe, Japan). Human GST-fusion CK2 α (1-391) or CK2 α' (1-350) was co-expressed with His-tagged human CK2 β (1-215) using baculovirus expression system. GST-CK2 α 1 was purified by using glutathione sepharose chromatography. Each chemical in DMSO at different concentrations was diluted fourfold with reaction buffer [20 mM HEPES (pH 7.5), 0.01% Triton X-100, 2 mM DTT]. For CK2 reactions, a combination of the compound, 1 μ M CK2tide, 5 mM MgCl₂, 5 μ M ATP in reaction buffer (0.020 cm³) were incubated with each CK2 in PP 384-well plates at room temperature for 1 h ($n = 2$). The reaction was terminated by addition of 0.060 cm³ of termination buffer (Carna Biosciences). Substrate and product were separated by electrophoretic means using the LabChip3000 system. The kinase reaction was evaluated by the product ratio, which was calculated from the peak heights of the substrate (S) and product (P): [P/(P+S)]. Inhibition data were calculated by comparing with no-enzyme controls for 100% inhibition and no-inhibitor reactions for 0% inhibition. IC₅₀ values were calculated using GraphPad Prism 5 software (GraphPad Software, Incorporated, La Jolla, CA, USA).

Acknowledgements

This work was supported by Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO), and Grants-in-Aid for Scientific Research and Platform for Drug Discovery, Informatics, and Structural Life Science from the MEXT, Japan. Z.H. and Y.S. are supported by the Kobayashi International Scholarship Foundation and by a JSPS Research Fellowship for Young Scientists, respectively.

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15. The reaction with 5 mol% of IPrAuNTf₂ prepared from IPrAuCl and AgNTf₂ proceeded smoothly to afford the dihydropyrazole **9a** in slightly lower yield (65%). As we previously reported,¹³ the use of silver salts alone is nonreactive for the three-component annulation. These results supported that the *in situ* generated active gold(I) species (IPrAuOTf or IPrAuNTf₂) is the real catalyst.
16. The 3-chloroaniline moiety of CX-4945, which locates at the similar position with that of R¹ or R² in pyrazolo[4,3-*b*]indoles **5**, has been suggested to fit well within a small hydrophobic region of CK2, see: ref 5g and 5h.

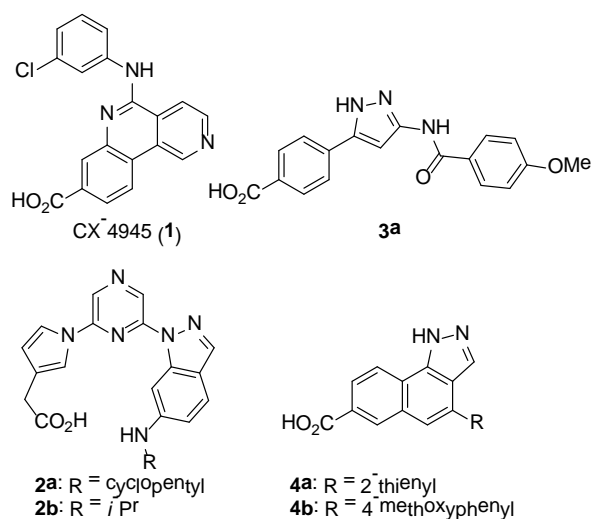


Figure 1. Structures of reported CK2 inhibitors

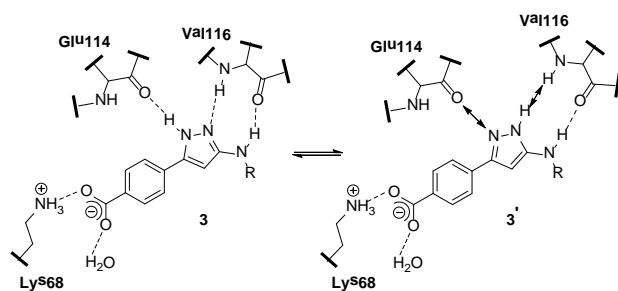


Figure 2. Plausible binding mode of phenylpyrazole **3** with CK2α

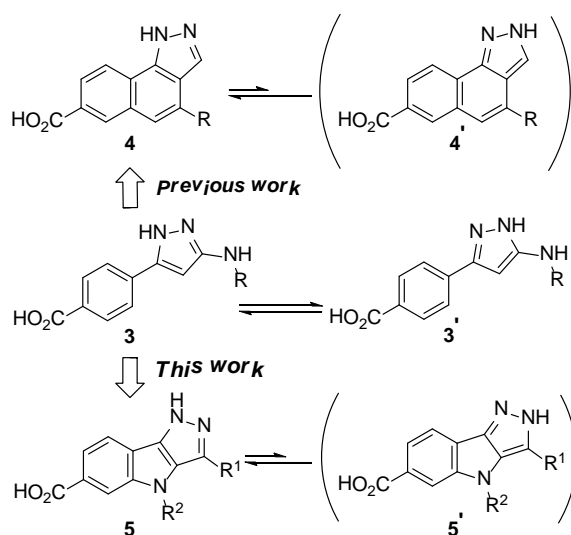
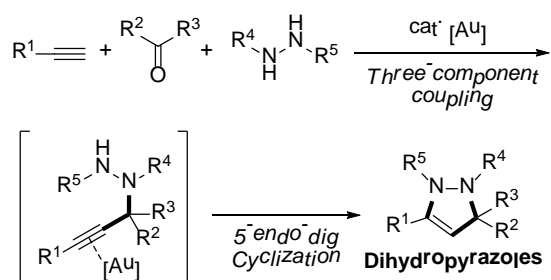
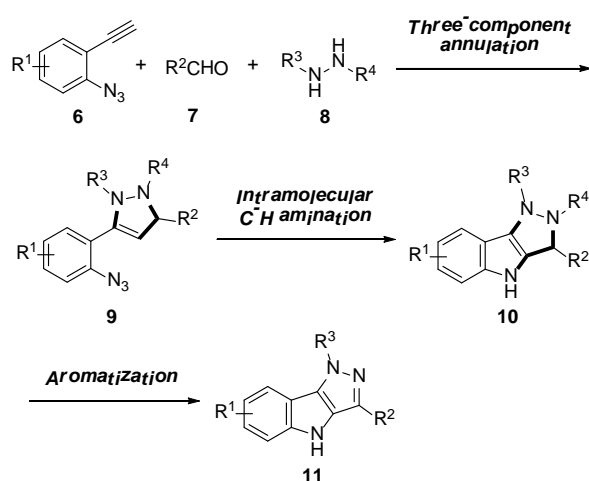


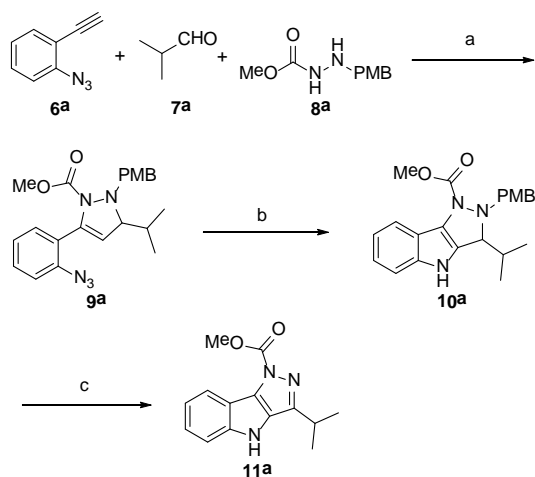
Figure 3. Phenylpyrazole-based CK2 inhibitor **3**, benzo[*g*]indazoles **4**, pyrazolo[4,3-*b*]indoles **5** and their tautomers **3'**, **4'** and **5'**



Scheme 1. Gold-catalysed three-component annulation reaction



Scheme 2. Synthetic strategy for the construction of the polysubstituted pyrazolo[4,3-*b*]indoles **11**



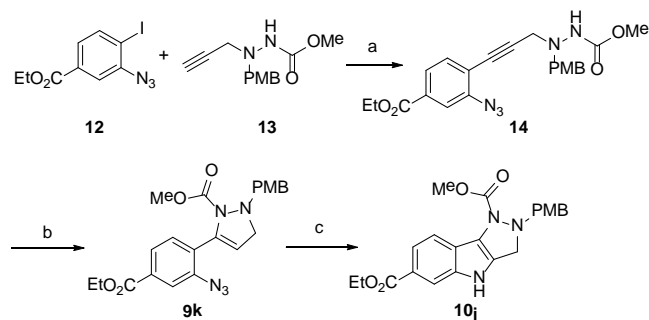
Scheme 3. Model experiments.

Reagents and conditions: (a) IPrAuCl/AgOTf (3 mol%), 1,2-DCE, 50 °C, 4 h, 73%; (b) RuCl₃·nH₂O (5 mol%), 1,2-dimethoxyethane (1,2-DME), 80 °C, 4 h, 62%; (c) CAN (2 eq.), MeCN/H₂O, rt, 12 h, 87%.

Table 1. Evaluation of reaction scope

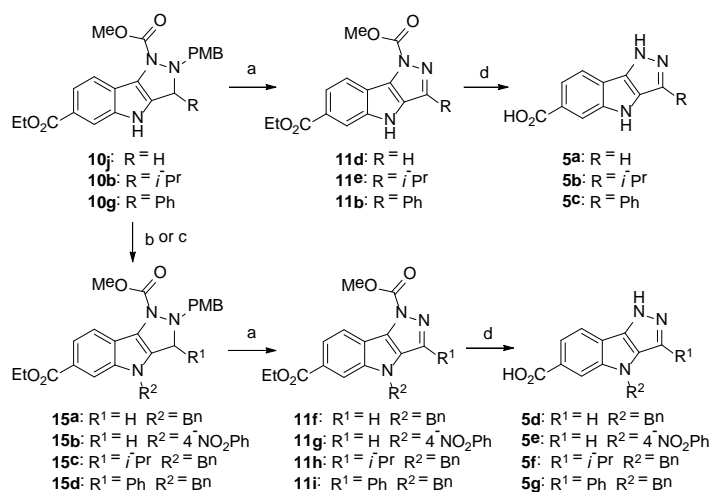
entry	dihydropyrazole 9^a	dihydropyrazolo[4,3- <i>b</i>]indole 10 and/or pyrazolo[4,3- <i>b</i>]indole 11^a	entry	dihydropyrazole 9^a	dihydropyrazolo[4,3- <i>b</i>]indole 10 and/or pyrazolo[4,3- <i>b</i>]indole 11^a
1			7		
2					
3			8		
4			9		
5			10		
6			11		

^a Isolated yields. ^b IPrAuCl/AgNTf₂ (3 mol%) were used as the catalyst. ^c The reaction was carried out at rt. ^d Cyclohexanone was used instead of an aldehyde. ^e The reaction was carried out in the absence of the Ru(III) catalyst in *o*-dichlorobenzene at 150 °C.



Scheme 4. Synthesis of the dihydropyrazole **9k** and dihydropyrazolo[4,3-*b*]indole **10j**.

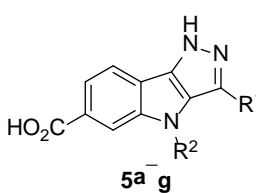
Reagents and conditions: (a) $\text{PdCl}_2(\text{PPh}_3)_2/\text{CuI}$ (5 mol%), Et_3N , THF, 40 °C, 5 h, 70%; (b) $\text{IPrAuCl}/\text{AgOTf}$ (3 mol%), 1,2-DCE, 50 °C, 4 h, 74%; (c) *o*-dichlorobenzene, 150 °C, 2 h, 43%.



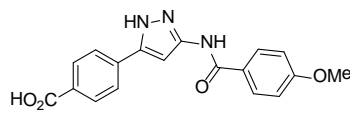
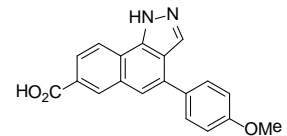
Scheme 5. Synthesis of CK2 inhibitor compounds **5a–g**.

Reagents and conditions: (a) CAN, MeCN/H₂O, rt; (b) NaH, BnBr, DMF, rt; (c) NaH, 1-fluoro-4-nitrobenzene, DMF, rt; (d) 3N NaOH, THF/MeOH, 60 °C.

Table 2. Evaluation of the CK2 inhibitory activities of the compounds



5a⁻ g

Compd	R ¹	R ²	IC ₅₀ [μM] ^a	
			CK2α	CK2α'
5a	H	H	0.785	0.652
5b	<i>i</i> -Pr	H	> 1.00	> 1.00
5c	Ph	H	0.0583	0.0280
5d	H	Bn	> 1.00	~ 1.00
5e	H	4-NO ₂ Ph	0.0581	0.0280
5f	<i>i</i> -Pr	Bn	> 1.00	> 1.00
5g	Ph	Bn	0.157	0.120
3a			0.0387	0.00551
4b			0.0392	0.0194

^a Inhibition values were determined by the CK2 kinase assay